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Frequency of *BCL-2/J_H* Translocations in Peripheral Blood of Follicular Lymphoma Patients

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Polymerase chain reaction (PCR) assays have been developed for follicular lymphoma-associated *BCL-2/J_H* translocations. Few data are available on the quantitation by PCR of these translocations in peripheral blood mononuclear cells (PBM) of follicular lymphoma (FL) patients. We report that only one of five studied FL patients had a high level of these translocations in the circulation, namely, about 35,000 translocations per 5×10^6 PBM. This patient was stable with an excellent performance status at the time of this assay; however, he died of leukemia 1 month later. Am. J. Hematol. 55:205–207, 1997.

Key words: follicular lymphoma; *BCL-2*; PCR; translocations

INTRODUCTION

About 85% of follicular lymphomas (FLs) contain the *BCL-2/J_H* translocation [t(14;18)(q32;q21)] between the *BCL-2* proto-oncogene and a J_H immunoglobulin gene region [1]. Although recent PCR methods for detecting these translocations in patients' bone marrow and peripheral blood [2,3] might allow monitoring of the effectiveness of treatment and the progress of the disease, their prognostic significance is controversial [4,5]. Also, as a complicating factor, healthy individuals often have PCR-detectable translocations in their peripheral blood at levels ranging from 1–1,000 translocations per 5×10^6 peripheral blood mononuclear cells (PBM) [6,7]. We found that one of five studied FL patients displayed the highest reported frequency of these PBM-associated translocations. In contrast, the other four patients had undetectable levels or translocation frequencies in the range common for the PBM of healthy individuals.

MATERIALS AND METHODS

Patients

Patient 1 (Lsp53) was a 62-year-old white male diagnosed with Stage IV follicular lymphoma in 1986. At the time of presentation, the patient had lymphadenopathy and bone marrow and skin involvement. He received eight cycles of CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone). Then he was treated for recurrence of his skin lesions with Pro Mace/Cyta BOM (cyclophosphamide, doxorubicin, etoposide, prednisone, cytosine arabinoside, bleomycin, vincristine, methotrexate), and was maintained on chlorambucil until January 1991. Two months after stopping the medication, he relapsed in the skin and had a partial response when chlorambucil was resumed. At the time of the blood sampling for PCR analysis (May 1996), his WBC count was 6,600 $\times 10^3/\mu l$; neutrophils, 75.8%; lymphocytes, 15.0%; monocytes, 6.9%; and his performance status was excellent. However, in June 1996 he was admitted with severe

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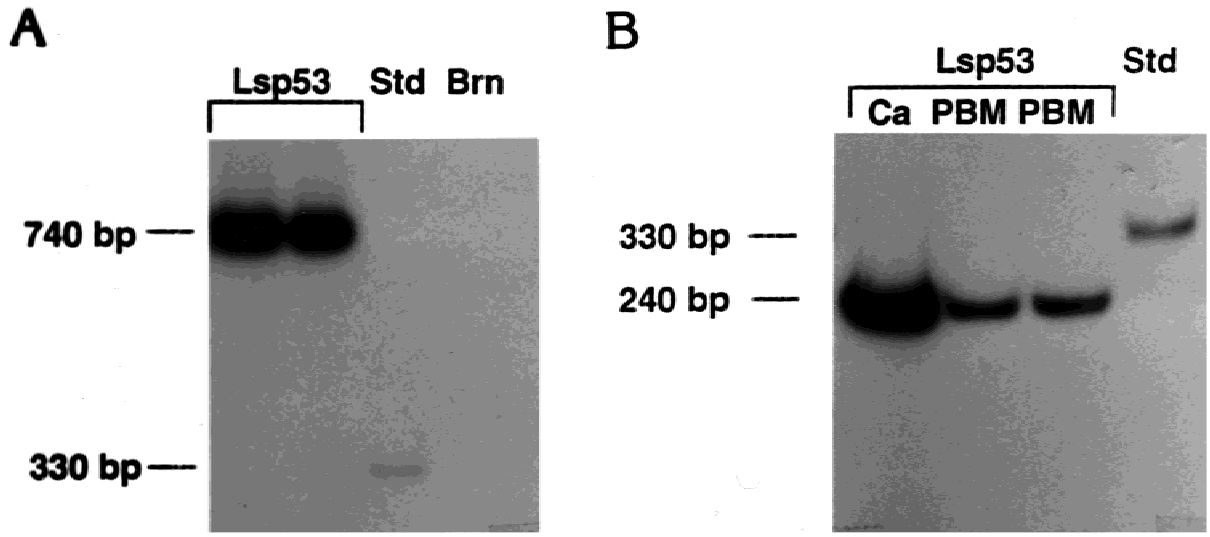

Fig. 1. PCR-amplified products from a *BCL-2/J_H* translocation in peripheral blood and in an archival lymphoma sample from an FL patient. A: 0.5 or 0.25 μg of Lsp53 PBM DNA; 4 copies from the *BCL2/J_H*-positive lymphoma cell line standard (Std.;3); 7 μg of translocation-negative brain DNA (Brn). B: 3.5 μg of Lsp53 lymphoma DNA (Ca) or 5 ng of Lsp53 PBM DNA, in duplicate, were used for PCR with the standard primers (A) or with the closer set of primers (B). In B, standard primers were used for the *BCL-2/J_H*-positive control (Std). The electrophoresed radiolabeled products were visualized by autoradiography.

anemia and thrombocytopenia. His WBC was 14,000/μl with 29% blasts in peripheral blood. Bone marrow aspirate and biopsy analyses were compatible with acute lymphoblastic leukemia. The patient's presentation was complicated by respiratory distress and pneumonia with infiltrates seen upon chest X-ray. The patient expired 2 days after admission to the hospital.

Patients 2–5 were all diagnosed with low-grade follicular lymphoma and were at Stages I, IV, IV, and III, respectively, at the time of blood sampling. Patient 2 (Lsp33) has been in remission and off therapy during the evaluation by PCR. Patient 3 (Lsp1) was receiving active therapy with chlorambucil for recurrent disease. Patient 4 (Lsp42) was recently diagnosed with FL and was receiving intravenous chemotherapy. Patient 5 (Lph15) was off therapy but with disease during the sampling for the PCR assay.

DNA Samples

Peripheral blood (10 ml) was obtained with informed consent from FL patients and PBM DNA isolated as previously described [7]. Extraction of DNA from paraffin-embedded tissues was done by standard techniques [8].

PCR

Semi-nested PCR for *BCL-2/J_H* translocations in the major breakpoint region (MBR) of the *BCL-2* gene [1] was conducted with previously described primers 639 and 667 for the first round of PCR and 639 and radiolabeled 641 for the second round [3]. Where indicated, we used primers 639 and 641 in the first round and a closer set of primers, 639 and radiolabeled primer 663 (5′-CTTTAGAGAGTTGCTTTACGTGG-3′), in the second round of PCR.

RESULTS

BCL-2/J_H Translocations in Circulating Blood Cells

DNA (3.5–35 μg) from peripheral blood of five FL patients was tested by PCR for MBR-type *BCL-2/J_H* translocations. The following levels of translocations were found per 5×10^6 PBM for patients Lsp15, Lsp42, Lsp1, Lsp33, and Lsp53: 0, 0, 2, 5, and about 35,000. To quantitate translocations in Lsp53 DNA, only 5–20 ng was amplified [3]. Figure 1A shows the product from Lsp53 obtained by PCR with the standard set of primers.

BCL-2/J_H Translocations in Tumor Samples

DNA was amplified from a section of paraffin-embedded lymphoma surgically removed from the skin 10 years ago from patient Lsp53. The closer set of primers was used for this PCR because of partial degradation of the tumor DNA. A product within the expected size range was seen from this tumor and from his analogously amplified PBM DNA (Fig. 1B). Tumor samples were also available from patients Lsp1 and LspC42. PCR amplification indicated that the former, but not the latter, had MBR-type *BCL-2/J_H* translocations.

DISCUSSION

Healthy adults frequency have low levels of *BCL-2/J_H* translocations in their peripheral blood [7]. We found an abnormally high level of the major type of these FL-associated translocations, MBR-type *BCL-2/J_H*-translocations, in only one of five FL patients. This patient's tumor-derived PCR product was the same size as that from his PBM DNA. Therefore, his high level of circulating translocation-containing cells were probably FL cells and not potentially pre-malignant cells of the type observed in about half of healthy adults [7].

Circulating lymphoma cells have been detected in patients with lymphoma using flow cytometry and PCR, and a correlation with treatment has been reported; however, the prognostic significance of these findings is still unclear [9,10]. In our study there was no correlation of tumor stage with PBM translocation level in the five FL patients. However, the extraordinarily high translocation level in one patient, whose clinical course suddenly worsened 1 month after the PCR analysis, may have been predictive for an aggressive transforamtion of his FL into leukemia.

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